Amendment to the Specification

Please amend the specification to add the following paragraph on page 1, after the title at line 7:

Cross Reference to Related Application

This application is the National Stage of International application PCT/US2004/031417 filed on September 24, 2004, and published as WO 2005/030949 A1, which claims the benefit of United States Provisional Patent Application Number 60/505,658, filed on September 24, 2003, now expired, each of the foregoing of which is incorporated by reference herein in its entirety.

Please amend the specification starting at page 2, line15, through page 4, line 8 (paragraph 8):

[0008] The present invention further provides:

- Recombinant KDPGal aldolase polypeptides having the ability to catalyze the condensation of pyruvate and E4P to from DAHP, containing at least one mutation that is X10V, X28L or X28M, X42T, X85A, X154F, or X196I; KDPGal aldolase polypeptides containing at least one mutation that is I10V, V28L or V28M, S42T, V85A, V154F, or F196I;
- KDPGal aldolase polypeptides having, apart from one of these mutations, the amino acid sequence of any one of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6, or an amino acid sequence at least 50% homologous to one of these; recombinant KDPGal aldolase polypeptides whose amino acid sequences are variants of a native KDPGal aldolase amino acid sequence;
- Nucleic acid encoding such a recombinant KDPGal aldolase polypeptide; vectors containing such nucleic acid;
- Enzymatic pathways capable of converting pyruvate and D-erythrose 4-phosphate (E4P) into 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP), by virtue of their having at least one KDPGal aldolase; such enzymatic pathways also capable of converting DAHP to DHQ by virtue of their having at least one DHQ synthase, and optionally at least one DHQ dehydratase, and further optionally at least one shikimate dehydrogenase;
- Methods for the production of shikimate or a shikimate intermediate, such as DAHP, DHQ, or DHS, by growing a recombinant host cell containing nucleic acid encoding at least one

KDPGal aldolase and at least one DHQ synthase, such that the cell expresses those enzymes. Methods for converting pyruvate and E4P to DAHP or a derivative of DAHP in vitro or in vivo/in cyto;

- The use of a recombinant KDPGal aldolase to produce DAHP from pyruvate and E4P; the use of a combination of recombinant KDPGal aldolase and DHQ synthase to produce DHQ.
- Process for preparing recombinant cells capable of expressing a KDPGal aldolase, and thus of converting pyruvate and E4P to DAHP by providing a host cell capable of synthesizing pyruvate and E4P, providing a vector containing a polynucleotide from which said host cell can express a KDPGal aldolase, and transforming said cell with said vector to produce a transformed cell, and, optionally, expressing the KDPGal aldolase, whereupon the cell converts pyruvate and E4P to DAHP.
- Recombinant cells prepared thereby;
- Processes for preparing DAHP or a derivative thereof, by providing (A) a supply of E4P and pyruvate, (B) a KDPGal aldolase, and optionally a DHQ synthase, (C) an aqueous medium; contacting the KDPGal aldolase with the E4P and pyruvate under conditions in which the KDPGal aldolase can catalyze the formation of DAHP therefrom, and optionally contacting the DAHP with the DHQ synthase under conditions in which the DHQ synthase can catalyze the formation of 3-dehydroquinate from the DAHP; and optionally recovering at least one of DAHP, DHQ, DHS, or a further-derivative thereof;
- In vivo embodiments of such methods, pathways, and cells further including a recombinant transketolase or a recombinant transaldolase;
- Kits containing a KDPGal aldolase preparation, with instructions for the use thereof to convert pyruvate and E4P to DAHP, and optionally with instructions for the conversion of said DAHP to at least one derivative thereof;
- Kits containing a cell capable of expressing a KDPGal aldolase, with instructions for the use thereof to convert pyruvate and E4P to DAHP, and optionally with instructions for the conversion of said DAHP to at least one derivative thereof;
- Kits containing nucleic acid from which a cell can express at least one KDPGal aldolase, with instructions for the use thereof to transform a cell to produce a transformed cell that is

capable of ~~onverting~~ converting pyruvate and E4P to DAHP, and optionally to at least one derivative thereof.

Please amend the specification on page 25, line 14:

Table [5] 6

Please amend the specification at page 8, lines 16-27 (paragraph 27),:

[0027] Just as a polypeptide may contain conservative amino acid substitution(s), a polynucleotide hereof may contain conservative codon substitution(s). A codon substitution is considered conservative if, when expressed, it produces a conservative amino acid substitution, as described above. Degenerate codon substitution, which results in no amino acid substitution, is also useful in polynucleotides according to the present invention. Thus, e.g., a polynucleotide encoding a selected polypeptide useful in an embodiment of the present invention may be mutated by ~~degerate~~ degenerate codon substitution in order to approximate the codon usage frequency exhibited by an expression host cell to be transformed therewith, or to otherwise improve the expression thereof.

Production of Shikimate and its Precursors

Please amend the specification at page 9, lines 6-14 (paragraph 29):

[0029] By catalyzing the reversible cleavage of KDPGal to pyruvate and D-glyceraldehyde 3-phosphate (G3P, Figure 2), KDPGal aldolase enables microbes (e.g., *E. coli*) to use D-galactonate as a sole carbon source. In one embodiment, *E. coli* dgoA-encoded KDPGal aldolase is ~~overexpresssed~~ overexpressed, partially purified, and incubated with pyruvate, D-erythrose 4-phosphate (E4P), 3-dehydroquinate synthase, and 3-dehydroquinate dehydratase to form 3-dehydroshikimate. Dehydratase-catalyzed dehydration of 3-dehydroquinate provides in product 3-dehydroshikimate a chromophore suitable for continuous spectrophotometric assay.

Please amend the specification at page 16, lines 5-14 (paragraph 53):

[0053] Random mutagenesis of dgoA gene is conducted using methods described by Cadwell and Joyce: Cadwell, R. C., Joyce, G. F., *PCR Meth. Appl.,* 1992, 2, 28-33. PCR is performed in a 100 μL reaction mixture containing 10 ng of dgoA fragment as template, 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 7 mM $MgCl_2$, 0.5 mM $MnCl_2$, 0.2 mM dATP, 0.2 mM dGTP, 1.0 mM dCTP, 1.0 mM dTTP, 50 μmol of each primer and 5 U Taq ~~polyerase~~ polymerase (Invitrogen). Conditions for PCR are as follows: one cycle of 4 min at 94° C., 22 cycles of 45 sec 94° C., 45 sec 45° C., 45 sec 72° C.; and one cycle of 10 min 25° C.

DNA Shuffling